methods that produce oils with shorter chain length and a higher degree of saturation and without pigments, with greater yield and efficiency. The present invention meets this need.

[0006] A polyurethane is a compound that comprises a carbamate (urethane) linkage. Typically, a polyurethane is a polymer of organic units. The polymer is prepared by the reaction of a first organic unit comprising an isocyanate moiety ($C(O)N\text{-}R_1\text{-}NC(O)$) and a second organic unit comprising a hydroxyl group ($HO\text{-}R_2\text{-}OH$). A polyurethane can be represented as $-[C(O)NH\text{-}R_1\text{-}NHC(O)\text{-}O\text{-}R_2\text{-}O]_m-$, wherein the subscript m is a number that denotes the number of monomers contained in the polymer. R_1 and R_2 can be the same or different, but are typically different. Polyurethanes are used in many different applications including both flexible and rigid materials. Polyurethanes are used in shoes, automobiles, airplanes, bushings, gaskets, adhesives, carpeting, spandex fibers, housing for electronics and the like.

SUMMARY OF THE INVENTION

[0006a] In a first aspect, the present invention refers to a method for using sucrose to produce an oil comprising triacylglycerides that comprise ricinoleic acid, or a product produced from the oil, the method comprising:

cultivating a cell of a recombinant oleaginous microalga of the species *Prototheca moriformis*, the cell comprising:

nucleic acids operable to enhance oleic acid production so as to elevate the substrate levels for an exogenous oleate 12-hydroxylase by expressing a product of an exogenous gene encoding an active stearoyl ACP desaturase and decreasing or eliminating the expression of an enzyme encoded by one or more endogenous genes that encode a fatty acid desaturase by directly targeting said endogenous gene(s) or RNA products of said gene(s); and

an exogenous nucleic acid operable to express the active oleate 12-hydroxylase, so as to synthesize the ricinoleic acid; and an exogenous nucleic acid operable to express an active invertase; and

feeding sucrose to the cell so as to produce the oil comprising triacylglycerides comprising ricinoleic acid, wherein the ricinoleic acid is detectable by gas chromatography-mass spectrometry.

[0006b] In a second aspect, the present invention refers to a microalga cell of the species *Prototheca moriformis*, the cell comprising:

nucleic acids operable to enhance oleic acid production so as to elevate the substrate levels for an exogenous oleate 12-hydroxylase by expressing a product of an exogenous gene encoding an active stearoyl ACP desaturase and decreasing or eliminating the expression of an enzyme encoded by one or more endogenous genes that encode a fatty acid desaturase by directly targeting said endogenous gene(s) or RNA products of said gene(s);

an exogenous gene encoding the active oleate 12-hydroxylase; and

an exogenous gene encoding an invertase so as to enable the cell to use sucrose to synthesize ricinoleic acid in an amount detectable by gas chromatography-mass spectrometry.

[0007] Illustrative ~~embodiments of the present invention~~examples provide oleaginous cells that produce altered glycerolipid profiles and products produced from the cells. Examples of oleagninous cells include microbial cells having a type II lipid biosynthesis pathway. ~~Embodiments~~Examples also feature natural oils, which are obtainable using the cells. ~~Embodiments~~Examples include recombinant cells expressing exogenous genes encoding proteins such as fatty acyl-ACP thioesterases. The present ~~invention~~disclosure also provides methods of making lipids and oil-based products, including fuels such as biodiesel, renewable diesel and jet fuel, food oils and chemicals from such cells.

[0008] In a first ~~aspect~~example, the present ~~invention~~disclosure provides a microalgal cell having a lipid profile that is at least 3% C8:0. In some cases, the lipid profile is at least 12% C8:0. In some ~~embodiments~~examples, the cell is a recombinant cell. In some cases, the recombinant cell comprises an exogenous gene encoding an acyl-ACP thioesterase protein that has hydrolysis activity towards fatty acyl-ACP substrates of chain length C8. In some ~~embodiments~~examples, the exogenous gene encodes a *Cuphea palustris* acyl-ACP thioesterase. In some cases, the cell is a *Prototheca* cell. In some cases, the cell is of a microalgal genus or species selected from microalgae identified in Table 1.

[0009] In a second ~~aspect~~example, the present ~~invention~~disclosure provides a microalgal cell having a lipid profile that is at least 4% C10:0. In some cases, the lipid profile is at least 18% C10:0. In some cases, the lipid profile is at least 20% C10:0. In some cases, the lipid content of the microalgal cell further comprises C12:0. In some cases, the ratio of C10:0 to C12:0 is at least 3:1. In some cases, the lipid content of the microalgal cell further comprises C14:0. In some

cases, the ratio of C10:0 to C14:0 is at least 10:1. In some ~~embodiments~~examples, the cell is a recombinant cell. In some cases, the recombinant cell comprises an exogenous gene encoding a fatty acyl-ACP thioesterase protein that has hydrolysis activity towards fatty acyl-ACP substrates of chain length C10. In some ~~embodiments~~examples, the exogenous gene encodes a fatty acyl-ACP thioesterase protein from a species selected from the group consisting of *Cuphea hookeriana* and *Ulmus americana*. In some ~~embodiments~~examples, the fatty acyl-ACP thioesterase protein is from a species selected from the group consisting of *Cuphea hookeriana* and *Ulmus americana*. In some case, the fatty acyl-ACP thioesterase gene is selected from the group consisting of a fatty acyl-ACP thioesterase gene from *Cuphea hookeriana* and *Ulmus americana* that has hydrolysis activity towards fatty acyl-ACP substrates of chain length C10.

[0010] In some cases, the cell is a *Prototheca* cell. In some ~~embodiments~~examples, the cell is of a microalgal genus or species selected from microalgae identified in Table 1.

[0011] In a third ~~aspect~~example, the present ~~invention~~disclosure provides a microalgal cell having a lipid profile that is at least 13% C12:0. In some cases, the cell is a recombinant cell. In some ~~embodiments~~examples, the recombinant cell comprises an exogenous gene encoding a fatty acyl-ACP thioesterase protein that has hydrolysis activity towards fatty acyl-ACP substrates of chain length C12. In some ~~embodiments~~examples, the fatty acyl-ACP thioesterase protein is from a species selected from the group consisting of *Umbellularia californica* and *Cinnamomum camphora*. In some cases, the fatty acyl-ACP thioesterase gene is selected from the group consisting of a fatty acyl-ACP thioesterase gene from *Umbellularia californica* and *Cinnamomum camphora* that has hydrolysis activity towards fatty acyl-ACP substrates of chain length C12. In some ~~embodiments~~examples, the cell is a *Prototheca* cell.

[0012] In a fourth ~~aspect~~example, the present ~~invention~~disclosure provides a microalgal cell having a lipid profile that is at least 10% C14:0. In some cases, the lipid profile is at least 35% C14:0. In some cases, the lipid content of the microalgal cell further comprises C12:0. In some cases, the ratio of C14:0 to C12:0 is at least 3:1. In some cases, the cell is a recombinant cell. In some ~~embodiments~~examples, the recombinant cell comprises an exogenous gene encoding a fatty acyl-ACP thioesterase protein that has hydrolysis activity towards fatty acyl-ACP substrates of chain length C14. In some ~~embodiments~~examples, the fatty acyl-ACP thioesterase protein is from a species selected from the group consisting of *Cinnamomum camphora* and *Ulmus americana*. In some cases, the fatty acyl-ACP thioesterase gene is selected from the group consisting of a fatty acyl-ACP thioesterase gene from *Cinnamomum camphora* and *Ulmus americana* that has hydrolysis activity toward fatty acyl-ACP substrates of chain length C14. In some cases,

the cell is a *Prototheca* cell. In some ~~embodiments~~examples, the cell is of a microalgal genus or species selected from microalgae identified in Table 1.

[0013] In a fifth ~~aspect~~example, the present ~~invention~~disclosure provides a microalgal cell having a lipid profile that is at least 15% C16:0. In some cases, the lipid profile is at least 39% C16:0. In some cases, the lipid profile is at least 67% C16:0. In some cases, the cell is a recombinant cell. In some ~~embodiments~~examples, the recombinant cell comprises an exogenous gene encoding a fatty acyl-ACP thioesterase protein that has hydrolysis activity towards fatty acyl-ACP substrates of chain length C16. In some ~~embodiments~~examples, the fatty acyl-ACP thioesterase protein is from a species selected from *Cuphea hookeriana* and *Ulmus Americana.* In some ~~embodiments~~examples, the recombinant cell comprises an exogenous gene encoding a fatty acyl-ACP thioesterase protein from a species selected from the group consisting of *Cuphea hookeriana* and *Ulmus americana* that have hydrolysis activity towards fatty acyl-ACP substrates of chain length C16. In some cases, the cell is a *Prototheca* cell. In some ~~embodiments~~examples, the microalgal cell further comprises an endogenous desaturase gene, wherein the endogenous desaturase gene has been mutated to encode a desaturase that is inactive or less active than the non-mutated desaturase, or wherein, said endogenous desaturase has been deleted from the microalgal cell genome.

[0014] In a sixth ~~aspect~~example, the present ~~invention~~disclosure provides a microalgal cell having a lipid profile that is at least 60% saturated fatty acids. In some cases the microalgal cell has a lipid profile that is at least 85% saturated fatty acids. In some cases, the cell is a recombinant cell. In some ~~embodiments~~examples, the recombinant cell comprises an exogenous gene encoding a fatty acyl-ACP thioesterase protein that has hydrolysis activity towards fatty acyl-ACP substrates of chain lengths C10-C16. In some cases, the cell is a *Prototheca* cell.

[0015] In a seventh ~~aspect~~example, the present ~~invention~~disclosure provides a microalgal cell having a lipid profile that is at least 19% C18:0. In some cases, the lipid profile is at least 27% C18:0. In some cases, the cell is a recombinant cell. In some ~~embodiments~~examples, the recombinant cell comprises an exogenous gene encoding a fatty acyl-ACP thioesterase protein that has hydrolysis activity towards fatty acyl-ACP substrates of chain length C18. In some ~~embodiments~~examples, the fatty acyl-ACP thioesterase protein is from a species selected from *Brassica napus*. In some ~~embodiments~~examples, the recombinant cell comprises an exogenous gene encoding a fatty acyl-ACP thioesterase protein from *Brassica napus* that has hydrolysis activity towards fatty acyl-ACP substrates of chain length C16.

[0016] In an eighth ~~aspect~~example, the present ~~invention~~disclosure provides a microalgal cell comprising an exogenous gene encoding a fatty acyl-ACP thioesterase protein from the group consisting of

Cuphea hookeriana, Umbellularia californica, Cinnamomun camphora, Cuphea palustris, Cuphea lanceolata, Iris germanica, Myristica fragrans, Garcinia mangostana, Elaeis guiniensis, Brassica napus, Ricinus communis and *Ulmus americana.*

[0017] In an ninth ~~aspect~~example, the present ~~invention~~disclosure provides a microalgal cell comprising an expression construct wherein the expression construct down-regulates the expression of an endogenous gene selected from the methods consisting of the endogenous gene has been mutated to encode a gene product that is inactive or less active than the non-mutated gene product, the endogenous gene has been deleted from the microalgal cell genome, and through a RNA-induced mechanism. In some cases, the method is a RNA-induced mechanism, such as RNAi, antisense and/or dsRNA. In some cases, the endogenous gene is a desaturase gene. In some ~~embodiments~~examples, the desaturase gene is a delta 12 fatty acid desaturase gene. In some cases, the cell is a *Prototheca* cell.

[0018] In an tenth ~~aspect~~example, the present ~~invention~~disclosure provides a microalgal cell as described in any of the above ~~aspects~~examples, wherein the microalgal cell is cultivated using stachyose, raffinose or melibiose as a carbon source.

[0019] In an eleventh ~~aspect~~example, the present ~~invention~~disclosure provides a microalgal cell having a lipid profile that is no more than 2% 18:2. In some cases, the present ~~invention~~disclosure provides a microalgal cell having a lipid profile that is no more than 7% 18:2.

[0020] In a twelfth ~~aspect~~example, the present ~~invention~~disclosure provides a method of making lipid. In one ~~embodiment~~example, the method comprises (a) cultivating a microalgal cell as discussed above until the cell is at least 20% lipid by dry weight; and (b) separating the lipid from water-soluble biomass components.

[0021] In a thirteenth ~~aspect~~example, the present ~~invention~~disclosure provides another method of making lipid. In one ~~embodiment~~example, the method comprises (a) cultivating a microalgal cell containing two different exogenous genes encoding two different acyl-ACP thioesterases, and (b) separating the lipid from water-soluble biomass components. In some cases, at least one of the exogenous genes encodes a fatty acyl-ACP thioesterase selected from the group consisting of the thioesterases identified in Table 4.

[0022] In a fourteenth ~~aspect~~example, the present ~~invention~~disclosure provides a method of making an oil-based product. In one ~~embodiment~~example, the method comprises (a) cultivating a microalgal cell as discussed above until the cell is at least 10% lipid by dry weight;(b) separating the lipid from the microalgal cell; (c) subjecting the lipid to at least one chemical reaction selected from the group consisting of: saponification; metathesis; acid hydrolysis; alkaline hydrolysis; enzymatic hydrolysis; catalytic hydrolysis; hot-compressed water hydrolysis; a catalytic

hydrolysis reaction wherein the lipid is split into glycerol and fatty acids; an amination reaction to produce fatty nitrogen compounds; an ozonolysis reaction to produce mono- and dibasic-acids; a triglyceride splitting reaction selected from the group consisting of enzymatic splitting and pressure splitting; a condensation reaction that follows a hydrolysis reaction; a hydroprocessing reaction; a hydroprocessing reaction and a deoxygenation reaction and/or a condensation reaction prior to or simultaneous with the hydroprocessing reaction; a gas removal reaction; a deoxygenation reaction selected from the group consisting of a hydrogenolysis reaction, hydrogenation, a consecutive hydrogenation-hydrogenolysis reaction, a consecutive hydrogenolysis-hydrogenation reaction, and a combined hydrogenation-hydrogenolysis reaction; a condensation reaction following a deoxygenation reaction; an esterification reaction; an interestification reaction; a transesterification reaction; a hydroxylation reaction; and a condensation reaction following a hydroxylation reaction; and (d) optionally isolating a product of the reaction from the other components, whereby an oil-based product is produced.

[0023] In some cases, the oil-based product is selected from soap or a fuel product. In some ~~embodiments~~examples, the oil-based product is a fuel product selected from the group consisting biodiesel, renewable diesel, and jet fuel. In some cases, the fuel product is biodiesel with one or more of the following attributes: (i) 0.025-0.3 mcg/g, preferably 0.05-0.244 mcg/g, total carotenoids; (ii) less than 0.005 mcg/g, preferably less than 0.003 mcg/g, lycopene; (iii) less than 0.005 mcg/g, preferably less than 0.003 mcg/g, beta carotene; (iv) 0.025-0.3 mcg/g, preferably 0.045-0.268 mcg/g, chlorophyll A; (v) 35-175 mcg/g, preferably 38.3-164 mcg/g, gamma tocopherol; (vi) less than 0.25% brassicasterol, campesterol, stignasterol, or beta-sitosterol; (vii) 225-350 mcg/g, preferably 249.6-325.3 mcg/g, total tocotrienols; (viii) 0.0025-0.05 mcg/g, preferably 0.003-0.039 mcg/g, lutein; or (ix) 50-300 mcg/g, preferably 60.8-261.7 mcg/g, tocopherols. In some cases, the fuel product is renewable diesel that has a T10-T90 of at least 20°C, 40°C or 60°C. In some cases, the fuel product is jet fuel that meets HRJ-5 and/or ASTM specification D1655.

[0024] In a fifteenth ~~aspect~~example, the present ~~invention~~disclosure provides a triglyceride oil comprising (a) a lipid profile of at least 1-5%, preferably at least 3%, C8:0, at least 2.5%, preferably at least 4%, C10:0, at least 10%, preferably at least 13%, C12:0, at least 10% C14:0, and/or at least 60% saturated fatty acids, and (b) one or more of the following attributes: (i) 0.025-0.3 mcg/g, preferably 0.05-0.244 mcg/g, total carotenoids; (ii) less than 0.005 mcg/g, preferably less than 0.003 mcg/g, lycopene; (iii) less than 0.005 mcg/g, preferably less than 0.003 mcg/g, beta carotene; (iv) 0.025-0.3 mcg/g, preferably 0.045-0.268 mcg/g, chlorophyll A; (v)

35-175 mcg/g, preferably 38.3-164 mcg/g, gamma tocopherol; (vi) less than 0.25% brassicasterol, campesterol, stignasterol, or beta-sitosterol; (vii) 225-350 mcg/g, preferably 249.6-325.3 mcg/g, total tocotrienols; (viii) 0.0025-0.05 mcg/g, preferably 0.003-0.039 mcg/g, lutein; or (ix) 50-300 mcg/g, preferably 60.8-261.7 mcg/g, tocopherols.

[0025] In a sixteenth ~~aspect~~example, the present ~~invention~~disclosure provides a triglyceride oil isolated from microalgae that has a C8:C10 fatty acid ratio of at least 5:1. In some ~~embodiments~~examples, the triglyceride oil is isolated from microalgal cell (*e.g.*, of the genus *Prototheca*), wherein the microalgal cell comprises an exogenous gene. In a related ~~aspect~~example, the present ~~invention~~disclosure provides a triglyceride oil isolated from microalgae with at least 60% saturated fatty acids.

[0026] In another related ~~aspect~~example, the present ~~invention~~disclosure provides a triglyceride oil isolated from microalgae having a lipid profile that has a C16:14 fatty acid ratio of about 2:1. In another related ~~aspect~~example, the present ~~invention~~disclosure provides a triglyceride oil produced by a microalgal cell, wherein the microalgal cell comprises an exogenous gene. In some cases, the microalgae is of the genus *Prototheca*.

[0027] In another related ~~aspect~~example, the present ~~invention~~disclosure provides a triglyceride oil isolated from microalgae having a lipid profile that has a C12:14 fatty acid ratio of about 3:1. In another related ~~aspect~~example, the present ~~invention~~disclosure provides a triglyceride oil produced by a microalgal cell, wherein the microalgal cell comprises an exogenous gene. In some cases, the microalgae is of the genus *Prototheca*.

[0028] In a seventeenth ~~aspect~~example, the present ~~invention~~disclosure provides a triglyceride oil comprising (a) a lipid profile of less than 1% <C12; between 1%-10%, preferably 2%-7%, C14:0; between 20%-35%, preferably 23%-30%, C16:0; between 5%-20%, preferably 7%-15%, C18:0; between 35%-60%, preferably 40%-55%, C18:1; and between 1%-20%, preferably 2%-15%, C18:2 fatty acids; and (b) one or more of the following attributes: (i) 0.025-0.3 mcg/g, preferably 0.05-0.244 mcg/g, total carotenoids; (ii) less than 0.005 mcg/g, preferably less than 0.003 mcg/g, lycopene; (iii) less than 0.005 mcg/g, preferably less than 0.003 mcg/g, beta carotene; (iv) 0.025-0.3 mcg/g, preferably 0.045-0.268 mcg/g, chlorophyll A; (v) 35-175 mcg/g, preferably 38.3-164 mcg/g, gamma tocopherol; (vi) less than 0.25% brassicasterol, campesterol, stignasterol, or beta-sitosterol; (vii) 225-350 mcg/g, preferably 249.6-325.3 mcg/g, total tocotrienols; (viii) 0.0025-0.05 mcg/g, preferably 0.003-0.039 mcg/g, lutein; or (ix) 50-300 mcg/g, preferably 60.8-261.7 mcg/g, tocopherols.

[0029] In some cases, the triglyceride oil is isolated from a microbe comprising one or more exogenous gene. In some ~~embodiments~~examples, the one or more exogenous gene encodes a fatty acyl-ACP thioesterase. In some cases, the fatty acyl-ACP thioesterase has hydrolysis

activity towards fatty acyl-ACP substrates of chain length C14. In some ~~embodiments~~examples, the microbe further comprising expression construct wherein the expression construct down-regulates the expression of an endogenous gene selected from the methods consisting of the endogenous gene has been mutated to encode a gene product that is inactive or less active than the non-mutated gene product, the endogenous gene has been deleted from the microalgal cell genome, and through a RNA-induced mechanism. In some ~~embodiments~~examples, the endogenous gene encodes a desaturase. In some cases, the desaturase is a stearoyl-acyl carrier protein desaturase (SAD) or a fatty acid desaturase (FAD).

[0030] In an eighteenth ~~aspect~~example, the present ~~invention~~disclosure provides a method of producing a triglyceride oil comprising a lipid profile of less than 1% <C12; between 2%-7% C14:0; between 23%-30% C16:0; between 7%-15% C18:0; between 40-55% C18:1; and between 2-15% C18:2 fatty acids, wherein the triglyceride oil is isolated from a microbe comprising one or more exogenous gene. In some cases, the triglyceride oil comprises a lipid profile of 3-5% C14:0; 25-27% C16:0; 10-15% C18:0; and 40-45% C18:1. In some ~~embodiments~~examples, the one or more exogenous gene encodes a fatty acyl-ACP thioesterase. In some cases, the fatty acyl-ACP thioesterase has hydrolysis activity towards fatty acyl-ACP substrates of chain length C14.

[0031] In a nineteenth ~~aspect~~example, the present ~~invention~~disclosure provides a microbial cell that produces ricinoleic acid. In some cases, the microbial cell is a microalgal cell. In some cases, the microbial cell and the microalgal cell comprises an exogenous gene that encodes a fatty acid hydroxylase. In some ~~embodiments~~examples, the fatty acid hydroxylase is an oleate 12-hydroxylase. In some cases, the fatty acid hydroxylase is from *Ricinus communis*. In some cases, the microbial cell is of the genus *Prototheca,* such as, for example *Prototheca moriformis.*

[0032] In a twentieth ~~aspect~~example, the present ~~invention~~disclosure provides a microalgal cell comprising an exogenous gene that encodes an alpha-galactosidase. In some cases, the microalgal cell comprising an exogenous gene that encodes an alpha-galactosidase wherein the alpha-galactosidase is secreted. In some ~~embodiments~~examples, the exogenous gene that encodes an alpha-galactosidase is from a genus selected from the group consisting of *Saccharomyces, Aspergillus and Cyamopsis.*

[0033] In a twentyfirst ~~aspect~~example, the present ~~invention~~disclosure provides a method of producing a lipid composition comprising the steps of: (a) cultivating a microalgal cell under heterotrophic conditions in the presence of a fixed carbon source, wherein the microalgal cell comprises an exogenous gene encoding an alpha-galactosidase and the fixed carbon source is selected from the group consisting of raffinose, stachyose and melibiose; (b) separating the lipid from the

non-lipid components; thereby producing a lipid composition. In some cases, the microalgal cell is of the genus *Prototheca.*

[0034] In a twentysecond ~~aspect~~example, the present ~~invention~~disclosure provides a microalgal cell comprising an exogenous gene that encodes a THIC enzyme. In some cases, the THIC enzyme is from a genus selected from the group consisting of *Coccomyxa, Arabidopsis,* and *Synechocystis*.

[0035] In another ~~aspect~~example, the present ~~invention~~disclosure provides a method of cultivating a microalgal cell in the absence of thiamine comprising expressing an exogenous gene that encodes a THIC enzyme. In some cases, the THIC enzyme is from a genus selected from the group consisting of *Coccomyxa, Arabidopsis,* and *Synechocystis.*

[0036] In other ~~aspects~~examples, the present ~~invention~~disclosure provides a microalgal cell as described herein, wherein said microalgal cell further comprises another exogenous gene selected from the group consisting of a sucrose invertase, an alpha-galactosidase, and a THIC enzyme.

[0037] Another ~~aspect~~example of this ~~invention~~disclosure provides a hydroxylated oil isolated from a microbial cell. In one ~~aspect~~example, the hydroxylated oil is isolated from a microbial cell, wherein the microbial cell is a microalgal cell (*e.g.,* of the genus *Prototheca*). In a further ~~aspect~~example, the hydroxylated oil is isolated from a *Prototheca moriformis* cell. In one ~~embodiment~~example, the hydroxylated oil is a hydroxylated triglyceride. The hydroxylated triglyceride may be chemically similar or identical to castor oil.

[0038] A further ~~aspect~~example of the ~~invention~~disclosure is a hydroxylated fatty acid. One ~~embodiment~~example of the hydroxylated fatty acid is ricinoleic acid.

[0039] In yet another ~~aspect~~example, the microbial hydroxylated oil or hydroxylated fatty acid is further hydroxylated. When ricinoleic acid is hydroxylated, a fatty acid containing two hydroxyl groups is provided.

[0040] Yet another ~~aspect~~example of the ~~invention~~disclosure provides a composition prepared by reacting a hydroxylated oil and/or a hydroxylated fatty acid with a compound that contains an isocyanate moiety to form a polyurethane.

[0041] Another ~~aspect~~example of the ~~invention~~disclosure provides a microalgal cell having a lipid profile that is at least 20% C18:2. In some cases, the microalgal cell has a lipid profile that is at least 30% C18:2. In some cases, the microalgal cell has a lipid profile that is at least 40% C18:2. In some cases, the microalgal cell has a lipid profile that is at least 50% C18:2.

[0042] Another ~~aspect~~example of the ~~invention~~disclosure provides a method of making a lipid, comprising: (a) cultivating a microalgal cell in a culture medium and monitoring the sugar concentration; (b) when the sugar concentration of the culture medium reaches less than about 1 gram per

liter, adding a first sugar solution to the culture medium at a continuous rate of between about 2 grams per hour per liter to about 10 grams per hour per liter for about 2 to about 24 hours; (c) adding a second sugar solution to the culture medium to maintain the sugar concentration of the culture medium at about 15 to about 20 grams per liter; and (d) isolating the lipid from the microalgal biomass. In some cases, the first sugar solution is added to the culture medium at a rate of about 4 grams of sucrose per hour per liter to about 6 grams of sucrose per hour per liter. In some cases, the first sugar solution is added to the culture medium at a rate of about 5.25 grams of sucrose per hour per liter. In some cases, the sugar is sucrose or glucose.

[0043] Another ~~aspect~~example of the ~~invention~~disclosure provides a microalgal cell having a lipid profile that is at least 10% C18:3. In some cases, the microalgal cell has a lipid profile that is at least 20% C18:3. In some cases, the microalgal cell has a lipid profile that is at least 30% C18:3. In some cases, the microalgal cell has a lipid profile is at least 40% C18:3. In some cases, the microalgal cell has a lipid profile is at least 50% C18:3.

[0044] Another ~~aspect~~example of the ~~invention~~disclosure provides a microorganism that produces a triglyceride comprising linoleic acid or linolenic acid, wherein the microorganism comprises a recombinant nucleic acid encoding a β-ketoacyl-ACP synthase II (KAS II) enzyme. In some cases, the microorganism further comprises a recombinant nucleic acid encoding a stearoyl ACP desaturase (SAD) enzyme. In some cases, the microorganism further comprises a recombinant nucleic acid encoding an oleate-specific thioesterase enzyme. In some cases, the microorganism further comprises a recombinant nucleic acid encoding a fatty acid desaturase (FAD) enzyme. In some cases, the microorganism further comprises a recombinant nucleic acid encoding a glycerolipid desaturase.

[0045] In the engineered microorganisms discussed above, the KAS II enzyme can comprise an amino acid sequence selected from the group consisting of SEQ ID NO: 175, SEQ ID NO: 176, SEQ ID NO: 177, SEQ ID NO: 178 and SEQ ID NO: 179. In some cases, the SAD enzyme can comprise an amino acid sequence selected from the group consisting of SEQ ID NO: 172, SEQ ID NO: 196, SEQ ID NO: 197, SEQ ID NO: 198, SEQ ID NO: 199, SEQ ID NO: 200, and SEQ ID NO: 201. In some cases, the oleate-specific thioesterase enzyme can comprise an amino acid sequence selected from the group consisting of SEQ ID NO: 195, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, and SEQ ID NO: 206. In some cases, the FAD enzyme can comprise an amino acid sequence seleted from the group consisting of SEQ ID NO: 181, SEQ ID NO: 182. SEQ ID NO: 183, SEQ ID NO: 184 and SEQ ID NO: 185. In some cases, the FAD enzyme is a Δ12 FAD enzyme

comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, and SEQ ID NO: 212. In some cases, the FAD enzyme is a Δ15 FAD enzyme comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, and SEQ ID NO: 221. In some cases, the glycerolipid desaturase is a ω-6 fatty acid desaturase, a ω-3 fatty acid desaturase, or a ω-6 oleate desaturase.

[0046] Any one of the engineered microorgansims discussed above can further comprise a recombinant nucleic acid encoding a sucrose utilization pathway enzyme. In some cases, the sucrose utilization pathway enzyme is a sucrose invertase.

[0047] In any one of the engineered microorganisms discussed above, the microorganism can be further engineered to increase the proportion of linoleic acid or linolenic acid relative to other fatty acids.

[0048] In any one of the engineered microorganisms discussed above, the microorganism can be further engineered to overexpress a thioesterase specific for or preferential to C18 substrates.

[0049] In any one of the engineered microorganisms discussed above, the microorganism can be futher engineered to decrease expression of a thioesterase specific for or preferential to a C8-C16 substrate.

[0050] In any one of the engineered microorganisms discussed above, the microorganism can be a microalgal cell. In some cases, the microalgal cell is of the genus *Prototheca*. In some cases, the microalgal cell is a *Prototheca moriformis* cell.

[0051] Another ~~aspect~~example of the ~~invention~~disclosure provides an oil produced by any one of the engineered microorganisms discussed above.

[0052] Another ~~aspect~~example of the ~~invention~~disclosure provides methods of producing the engineered microorganisms discussed above by introducing into the microorganisms one or more of the recombinant nucleic acids to produce triglycerides comprising linoleic acid or linolenic acid.

[0053] Another ~~aspect~~example of the ~~invention~~disclosure provides a method for producing a natural oil comprising triacylglycerides, or a product produced from the natural oil, in which the method comprises (i) cultivating a cell of a recombinant microorganism, the cell comprising recombinant nucleic acids operable to (a) decrease or eliminate the expression of an enzyme encoded by one or more genes that encode a β-ketoacyl-ACP synthase I, β-ketoacyl-ACP synthase II, stearoyl ACP desaturase, fatty acid desaturase, or acyl-ACP thioesterase, and optionally wherein the cell comprises recombinant nucleic acids operable to decrease or

ACP desaturase by RNA interference. In some cases, the oil produced has a fatty acid profile characterized by an increase in C18:0 fatty acids. In some cases, the oil produced is characterized by a fatty acid profile having at least 50, 60, 70, 80, or 90% C18:0. In some cases, the oil produced is characterized by a fatty acid profile having at least 50-75% C18:0. In some cases, the oil produced is further characterized by a fatty acid profile having at least 20-40% C18:1. In some cases, the cell comprises recombinant nucleic acids operable to decrease or eliminate the expression of two copies of a gene encoding a β-ketoacyl-ACP synthase I, β-ketoacyl-ACP synthase II, stearoyl ACP desaturase, or fatty acid desaturase. In some cases, the nucleic acids are operable to express a product of a fatty acid desaturase exogenous gene encoding an active a ω-3 fatty acid desaturase and/or a ω-6 oleate desaturase. In some cases, the oil produced has a fatty acid profile characterized by an elevated level of linoleic acid, linolenic acid, or both. In some cases, the fatty acid profile of the oil is characterized by having at least 10, 20, 30, 40, or 50% linoleic acid, linolenic acid, or both. In some cases, the further processing of the oil comprises one or more of refining, bleaching, deodorizing, metathesis, transesterification, hydrogenation, hydrolysis, hydrogenation, deoxygenation, hydrocracking, isomerization, hydrolxylation, interesterification, amidation, sulfonation, and sufurization. In some cases, the oil is processed to create a food oil, fatty acids, a fatty alcohol, a lubricant, a soap, a fatty acid ester, a fatty acid ethoxylate, a fatty amine, an akyl chloride, a fatty alchohol ethoxylate, a fatty alcohol sulfate, a fatty acid alkanolamide, a sulfonated oil, a sulfurized oil, diesel fuel, jet fuel, gasoline, fuel blendstock, fuel additive, lubricant additive, or coating.

[0057] Another ~~aspect~~example of the ~~invention~~disclosure provides natural oil obtainable by the methods discussed above.

[0058] Another ~~aspect~~example of the ~~invention~~disclosure provides a product made from the natural oil discussed above. In some cases, the product comprises a food oil, fatty acids, a fatty alcohol, a lubricant, a soap, a fatty acid ester, a fatty acid ethoxylate, a fatty amine, an akyl chloride, a fatty alchohol ethoxylate, a fatty alcohol sulfate, a fatty acid alkanolamide, a sulfonated oil, a sulfurized oil, diesel fuel, jet fuel, gasoline, fuel blendstock, fuel additive, chemical additive, or coating.

[0059] Another ~~aspect~~example of the ~~invention~~disclosure provides a recombinant cell comprising recombinant nucleic acids operable to (a) decrease or eliminate the expression of an enzyme encoded by one or more genes that encode a β-ketoacyl-ACP synthase I, β-ketoacyl-ACP synthase II, stearoyl ACP desaturase, fatty acid desaturase, or acyl-ACP thioesterase, and optionally wherein the cell comprises recombinant nucleic acids operable to decrease or

cases, the oil produced is characterized by a fatty acid profile having at least 50-75% C18:0. In some cases, the oil produced is further characterized by a fatty acid profile having at least 20-40% C18:1. In some cases, the cell comprises recombinant nucleic acids operable to decrease or eliminate the expression of two copies of a gene encoding a β-ketoacyl-ACP synthase I, β-ketoacyl-ACP synthase II, stearoyl ACP desaturase, or fatty acid desaturase. In some cases, the nucleic acids are operable to express a product of a fatty acid desaturase exogenous gene encoding an active a ω-3 fatty acid desaturase and/or a ω-6 oleate desaturase. In some cases, the oil produced has a fatty acid profile characterized by an elevated level of linoleic acid, linolenic acid, or both. In some cases, the fatty acid profile of the oil is characterized by having at least 10, 20, 30, 40, or 50% linoleic acid, linolenic acid, or both.

[0063] Another ~~aspect~~example of the ~~invention~~disclosure provides a natural oil or oil-containing product produced from the cells described above.

[0064] Another ~~aspect~~example of the ~~invention~~disclosure provides a method for producing a natural oil comprising triacylglycerides that comprise ricinoleic acid, or a product produced from the natural oil, the method comprising cultivating a cell of a recombinant microorganism, the cell comprising recombinant nucleic acids operable to express a product of an exogenous gene encoding an active oleate 12-hydroxylase, so as to synthesize the ricinoleic acid.

[0065] In some cases, the microorganism has a type II fatty acid biosynthesis pathway. In some cases, the microorganism is a microalga. In some cases, the microalga is an obligate heterotroph. In some cases, the microalga is a species of Prototheca. In some cases, the microalga is *Prototheca wickerhamii, Prototheca stagnora, Prototheca portoricensis, Prototheca moriformis,* or *Prototheca zopfii*. In some cases, the microalga is *Chlorella kessleri, Chlorella luteoviridis Chlorella protothecoides,* or *Chlorella vulgaris*. In some cases, the cell is a recombinant cell expressing an active sucrose invertase. In some cases, the cultivating is heterotrophic. In some cases, the cell produces at least 40, 50, 60, 70, 80, or 90% oleic acid absent the recombinant nucleic acids operable to express a product of an exogenous gene encoding an active oleate 12-hydroxylase. In some cases, the cell further comprises recombinant nucleic acids operable to enhance oleic acid production so as to elevate the substrate levels for the oleate 12-hydroxylase. In some cases, the cell comprises recombinant nucleic acids operable to (a) express a product of an exogenous gene encoding an active stearoyl ACP desaturase and decrease or eliminate the expression of an enzyme encoded by one or more genes that encode a fatty acid desaturase; or (b) express a product of an exogenous gene encoding an active β-ketoacyl-ACP synthase I and express a product of an exogenous gene encoding an active acyl-ACP thioesterase.

[0066] Another ~~aspect~~example of the ~~invention~~disclosure provides a product produced according to any of the methods discussed above.

[0067] Another ~~aspect~~example of the ~~invention~~disclosure provides a microorganism cell comprising recombinant nucleic acids operable to express a product of an exogenous gene encoding an active oleate 12-hydroxylase, so as to synthesize ricinoleic acid.

[0068] In some cases, the microorganism has a type II fatty acid biosynthesis pathway. In some cases, the microorganism is a microalga. In some cases, the microalga is an obligate heterotroph. In some cases, the microalga is a species of Prototheca. In some cases, the microalga is *Prototheca wickerhamii, Prototheca stagnora, Prototheca portoricensis, Prototheca moriformis,* or *Prototheca zopfii*. In some cases, the microalga is *Chlorella kessleri, Chlorella luteoviridis Chlorella protothecoides,* or *Chlorella vulgaris*. In some cases, the cell is a recombinant cell expressing an active sucrose invertase. In some cases, the cell is capable of heterotrophic growth. In some cases, the cell produces at least 40, 50, 60, 70, 80, or 90% oleic acid absent the recombinant nucleic acids operable to express a product of an exogenous gene encoding an active oleate 12-hydroxylase. In some cases, the cell further comprises recombinant nucleic acids operable to enhance oleic acid production so as to elevate the substrate levels for the oleate 12-hydroxylase. In some cases, the cell comprises recombinant nucleic acids operable to (a) express a product of an exogenous gene encoding an active stearoyl ACP desaturase and decrease or eliminate the expression of an enzyme encoded by one or more genes that encode a fatty acid desaturase; or (b) express a product of an exogenous gene encoding an active β-ketoacyl-ACP synthase I and express a product of an exogenous gene encoding an active acyl-ACP thioesterase.

[0069] Another ~~aspect~~example of the present ~~invention~~disclosure provides a food comprising an oil as discussed above.

[0070] These and other aspects and embodiments of the invention are described in the accompanying drawing, a brief description of which immediately follows, the detailed description of the invention below, and are exemplified in the examples below. Any or all of the features discussed above and throughout the application can be combined in various embodiments of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0071] Figure 1 shows a chromatogram of renewable diesel produced from *Prototheca* triglyceride oil.

[0072] Figure 2 shows GC retention times of a representative positive transgenic clone compared to the ricinoleic acid standard and a wildtype control.

WHAT IS CLAIMED IS:

1. A method for producing a natural oil comprising triacylglycerides, or a product produced from the natural oil, the method comprising:

cultivating a cell of a recombinant microorganism, the cell comprising recombinant nucleic acids operable to

(a) decrease or eliminate the expression of an enzyme encoded by one or more genes that encode a β-ketoacyl-ACP synthase I, β-ketoacyl-ACP synthase II, stearoyl ACP desaturase, fatty acid desaturase, or acyl-ACP thioesterase, and optionally wherein the cell comprises recombinant nucleic acids operable to decrease or eliminate the expression of two copies of a gene encoding a β-ketoacyl-ACP synthase I, β-ketoacyl-ACP synthase II, stearoyl ACP desaturase, or fatty acid desaturase, or acyl-ACP thioesterase; or

(b) express a product of an exogenous gene encoding an active β-ketoacyl-ACP synthase I, β-ketoacyl-ACP synthase II, stearoyl ACP desaturase, or fatty acid desaturase, or acyl-ACP thioesterase; or

(c) decrease or eliminate the expression of an enzyme encoded by one or more genes that encode a β-ketoacyl-ACP synthase I or β-ketoacyl-ACP synthase II, and express a product of an exogenous gene encoding an active stearoyl ACP desaturase, fatty acid desaturase, or acyl-ACP thioesterase; or

(d) decrease or eliminate the expression of an enzyme encoded by one or more genes that encode a stearoyl ACP desaturase or fatty acid desaturase, and express a product of an exogenous gene encoding an active β-ketoacyl-ACP synthase I, β-ketoacyl-ACP synthase II, or acyl-ACP thioesterase; and

recovering the natural oil from the cell, and optionally further processing the natural oil to produce a food, fuel, or chemical product,

wherein the natural oil has an altered fatty acid profile due to the recombinant nucleic acids.

2. The method of claim 1, wherein the microorganism synthesizes fatty acids through a type II fatty acid biosynthesis pathway.

3. The method of claim 2, wherein the microorganism is a microalga.

4. The method of claim 3, wherein the microalga is an obligate heterotroph.

5. The method of claim 2, wherein the microalga is a species of *Prototheca* or Chlorella.

6. The method of claim 5, wherein the microalga is *Prototheca wickerhamii, Prototheca stagnora, Prototheca portoricensis, Prototheca moriformis,* or *Prototheca zopfii.*

7. The method of claim 2, wherein the microalga is *Chlorella kessleri, Chlorella luteoviridis Chlorella protothecoides,* or *Chlorella vulgaris.*

8. The method of any of claims 1-7, wherein the cell is a recombinant cell expressing an active sucrose invertase.

9. The method of any of claims 1-8, wherein the cultivating is heterotrophic.

10. The method of any of claims 1-9, wherein the fatty acid desaturase is one or more of a ω-6 fatty acid desaturase, a ω-3 fatty acid desaturase, or a ω-6 oleate desaturase, or a delta 12 fatty acid desaturase.

11. The method of any of claims 1-10, wherein the cell is cultivated so as to comprise at least 50%, at least 60%, at least 70%, or 50 to 90% triglyceride by dry cell weight.

12. The method of any of claims 1-11, wherein the oil comprises less than 500, 50, or 5 ppm of colored molecules.

13. The method of any of claims 1-12, wherein the recombinant nucleic acids are stably integrated.

14. The method of claim 13, wherein the recombinant nucleic acids are stably integrated into the chromosome of the microorganism.

15. The method of claim 14, further comprising at least one selectable marker.

16. The method of any of claims 1-15, wherein the cell comprises recombinant nucleic acids operable to decrease or eliminate the expression of an enzyme through expression of antisense, RNAi, or dsRNA targeting the transcript of a gene encoding for the enzyme.

17. The method of any of claims 1-16, wherein the decrease or eliminatation of the expression of an enzyme encoded by one or more genes that encode a β-ketoacyl-ACP synthase I, β-ketoacyl-ACP synthase II, stearoyl ACP desaturase, or fatty acid desaturase is due to the interruption or replacement of the one or more genes with one or more genes encoding an active β-ketoacyl-ACP synthase I, β-ketoacyl-ACP synthase II, stearoyl ACP desaturase, or fatty acid desaturase, or acyl-ACP thioesterase.

18. The method of any of claims 1-17, wherein the recombinant cell further comprises an exogenous gene encoding an oleate 12-hydroxylase, so as to synthesize ricinoleic acid.

19. The method of any of claims 1-18, wherein the recombinant cell comprises nucleic acids operable to decrease or eliminate the expression of a β-ketoacyl-ACP synthase II encoded by a KASII gene, and to express a product of an exogenous gene encoding an acyl-ACP thioesterase.

20. The method of claim 19, wherein the cell produces an oil with a fatty acid profile characterized by having at least 40, 50, 60, 70, or 80% C16 fatty acids.

21. The method of claim 20, wherein the cell produces an oil with a fatty acid profile characterized by having at least 50-75% C16:0

22. The method of claim 20 or 21, wherein the cell produces an oil with a fatty acid profile further characterized by having at least 20-40% C18:1

23. The method of any of claims 19-22, wherein the exogenous gene encoding an acyl-ACP thioesterase produces an active acyl-ACP thioesterase having greater activity in hydrolysis of C8-C16 fatty acyl chains than a native acyl-ACP-thioestearase of the cell.

24. The method of any of claims 19-23, wherein the exogenous gene encoding an acyl-ACP thioesterase interrupts the KASII gene.

25. The method of any of claims 1-18, wherein the recombinant cell comprises nucleic acids operable to decrease or eliminate the expression of an enzyme encoded by one or more genes that encode a β-ketoacyl-ACP synthase I.

26. The method of claim 25, wherein the oil produced has a fatty acid profile characterized by a shorter mean fatty acid chain length as a result of the recombinant nucleic acids.

27. The method of any of claims 1-18, wherein the recombinant cell comprises nucleic acids operable to decrease or eliminate the expression of a fatty acid destaurase encoded by at least one FAD gene and express a product of a stearoyl-ACP desaturase exogenous gene encoding an active stearoyl ACP desaturase.

28. The method of claim 27, wherein nucleic acids are operable to decrease or eliminate the expression of a fatty acid destaurase encoded by multiple copies of a fatty acid desaturase gene.

29. The method of claim 27 or 28, wherein the Stearoyl-ACP desaturase exogenous gene is recombined into a locus within the coding region of the fatty acid desaturase gene.

30. The method of any of claims 27-29, wherein the oil produced has a fatty acid profile having elevated oleic acid.

31. The method of claim 30, wherein oleic acid comprises at least 50, 60, 70, 80, or 90% of the fatty acids.

32. The method of any of claims 1-18, wherein the recombinant cell comprises nucleic acids operable to express a product of a β-ketoacyl-ACP synthase II exogenous gene encoding an active β-ketoacyl-ACP synthase II.

33. The method of claim 32, wherein the oil produced is characterized by a fatty acid profile elevated in C18:1 fatty acids and reduced in C16 fatty acids as a result of the recombinant nucleic acids.A method of any of claims 1-18, wherein the recombinant cell comprises nucleic acids operable to decrease or eliminate the expression of an enzyme encoded by one or more genes that encode a stearoyl ACP desaturase by RNA interference.

34. The method according to claim 33, wherein the oil produced has a fatty acid profile characterized by an increase in C18:0 fatty acids.

35. The method of claim 34, wherein the oil produced is characterized by a fatty acid profile having at least 50, 60, 70, 80, or 90% C18:0.

36. The method of claim 35, wherein the oil produced is characterized by a fatty acid profile having at least 50-75% C18:0.

37. The method of anyd of claims 34- 36, wherein the oil produced is further characterized by a fatty acid profile having at least 20-40% C18:1.

38. The method of any of claims 1-18, wherein the cell comprises recombinant nucleic acids operable to decrease or eliminate the expression of two copies of a gene encoding a β-ketoacyl-ACP synthase I, β-ketoacyl-ACP synthase II, stearoyl ACP desaturase, or fatty acid desaturase.

39. The method for any of claims 1-18, operable to express a product of a fatty acid desaturase exogenous gene encoding an active a ω-3 fatty acid desaturase and/or a ω-6 oleate desaturase.

40. The method of claim 39, wherein the oil produced has a fatty acid profile characterized by an elevated level of linolcic acid, linolenic acid, or both.

41. The method of claim 35, wherein fatty acid profile of the oil is characterized by having at least 10, 20, 30, 40, or 50% linoleic acid, linolenic acid, or both.

42. The method of any of clams 1-41, wherein the further processing of the oil comprises one or more of refining, bleaching, deodorizing, metathesis, transesterification, hydrogenation, hydrolysis, hydrogenation, deoxygenation, hydrocracking, isomerization, hydrolxylation, interesterification, amidation, sulfonation, and sufurization.

43. The method of any of 1-42, wherein the oil is processed to create a food oil, fatty acids, a fatty alcohol, a lubricant, a soap, a fatty acid ester, a fatty acid ethoxylate, a fatty amine, an alkyl chloride, a fatty alchohol ethoxylate, a fatty alcohol sulfate, a fatty acid alkanolamide, a sulfonated oil, a sulfurized oil, diesel fuel, jet fuel, gasoline, fuel blendstock, fuel additive, lubricant additive, or coating.

44. A natural oil obtainable by the method of any of claims 1-43.

45. A product made from the natural oil of claim 44.

46. The product of claim 45comprising a food oil, fatty acids, a fatty alcohol, a lubricant, a soap, a fatty acid ester, a fatty acid ethoxylate, a fatty amine, an akyl chloride, a fatty alchohol ethoxylate, a fatty alcohol sulfate, a fatty acid alkanolamide, a sulfonated oil, a sulfurized oil, diesel fuel, jet fuel, gasoline, fuel blendstock, fuel additive, chemical additive, or coating.

47. A recombinant cell comprising recombinant nucleic acids operable to

(a) decrease or eliminate the expression of an enzyme encoded by one or more genes that encode a β-ketoacyl-ACP synthase I, β-ketoacyl-ACP synthase II, stearoyl ACP desaturase, fatty acid desaturase, or acyl-ACP thioesterase, and optionally wherein the cell comprises recombinant nucleic acids operable to decrease or eliminate the expression of two copies of a gene encoding a β-ketoacyl-ACP synthase I, β-ketoacyl-ACP synthase II, stearoyl ACP desaturase, or fatty acid desaturase, or acyl-ACP thioesterase; or

(b) express a product of an exogenous gene encoding an active β-ketoacyl-ACP synthase I, β-ketoacyl-ACP synthase II, stearoyl ACP desaturase, or fatty acid desaturase, or acyl-ACP thioesterase; or

(c) decrease or eliminate the expression of an enzyme encoded by one or more genes that encode a β-ketoacyl-ACP synthase I or β-ketoacyl-ACP synthase II, and express a product of an exogenous gene encoding an active stearoyl ACP desaturase, fatty acid desaturase, or acyl-ACP thioesterase; or

(d) decrease or eliminate the expression of an enzyme encoded by one or more genes that encode a stearoyl ACP desaturase or fatty acid desaturase, and express a product of an exogenous gene encoding an active β-ketoacyl-ACP synthase I, β-ketoacyl-ACP synthase II, or acyl-ACP thioesterase.

48. The cell of claim 46, wherein the microorganism synthesizes fatty acids through a type II fatty acid biosynthesis pathway.

49. The cell of claim 48, wherein the microorganism is a microalga.

50. The cell of claim 49, wherein the microalga is an obligate heterotroph.

51. The cell of claim 50, wherein the microalga is a species of *Prototheca* or Chlorella.

52. The cell of claim 51, wherein the microalga is *Prototheca wickerhamii, Prototheca stagnora, Prototheca portoricensis, Prototheca moriformis,* or *Prototheca zopfii.*

53. The cell of claim 51, wherein the microalga is *Chlorella kessleri, Chlorella luteoviridis Chlorella protothecoides,* or *Chlorella vulgaris.*

54. The cell of any of claims 46-53, wherein the cell is a recombinant cell expressing an active sucrose invertase.

55. The cell of any of claims 46-54, wherein the cell is capable of heterotrophic growth.

56. The cell of any of claims 46-55, wherein the fatty acid desaturase is one or more of a ω-6 fatty acid desaturase, a ω-3 fatty acid desaturase, or a ω-6 oleate desaturase, or a delta 12 fatty acid desaturase.

57. The cell of any of claims 46-56, wherein the cell is capable of beiong cultivated so as to comprise between at least 50%, at least 60%, at least 70%, or 50 and 90% triglyceride by dry cell weight.

58. The cell of any of claims 46-57, wherein the recombinant nucleic acids are stably integrated.

59. The cell of claim 58, wherein the recombinant nucleic acids are stably integrated into the chromosome of the microorganism.

60. The cell of claim 59, further comprising at least one selectable marker.

61. The cell of any of claims 46-60, wherein the cell comprises recombinant nucleic acids operable to decrease or eliminate the expression of an enzyme through expression of antisense, RNAi, or dsRNA targeting the transcript of a gene encoding for the enzyme.

62. The cell of any of claims 46-61, wherein the decrease or eliminatation of the expression of an enzyme encoded by one or more genes that encode a β-ketoacyl-ACP synthase I, β-ketoacyl-ACP synthase II, stearoyl ACP desaturase, or fatty acid desaturase is due to the interruption or replacement of the one or more genes with one or more genes encoding an active β-ketoacyl-ACP synthase I, β-ketoacyl-ACP synthase II, stearoyl ACP desaturase, or fatty acid desaturase, or acyl-ACP thioesterase.

63. The cell of any of claims 46-62, wherein the recombinant cell further comprises an exogenous gene encoding an oleate 12-hydroxylase, so as to synthesize ricinoleic acid.

64. The cell of any of claims 46-62, wherein the recombinant cell comprises nucleic acids operable to decrease or eliminate the expression of a β-ketoacyl-ACP synthase II encoded by a KASII gene, and to express a product of an exogenous gene encoding an acyl-ACP thioesterase.

65. The cell of claim 64, wherein the cell produces an oil with a fatty acid profile characterized by having at least 40, 50, 60, 70, or 80% C16 fatty acids.

66. The cell of claim 65, wherein the cell produces an oil with a fatty acid profile characterized by having at least 50-75% C16:0

67. The cell of claim 66, wherein the cell produces an oil with a fatty acid profile further characterized by having at least 20-40% C18:1

68. The cell of claim 64 or 65, wherein the exogenous gene encoding an acyl-ACP thioesterase produces an active acyl-ACP thioesterase having greater activity in hydrolysis of C8-C16 fatty acyl chains than a native acyl-ACP-thioestearase of the cell.

69. The cell of any of claims 64-66, wherein the exogenous gene encoding an acyl-ACP thioesterase interrupts the KASII gene.

70. The cell of any of claims 46-62, wherein the recombinant cell comprises nucleic acids operable to decrease or eliminate the expression of an enzyme encoded by one or more genes that encode a β-ketoacyl-ACP synthase I.

71. The cell of claim 70, wherein the oil produced has a fatty acid profile characterized by a shorter mean fatty acid chain length as a result of the recombinant nucleic acids.

72. The cell of any of claims 46-62, wherein the recombinant cell comprises nucleic acids operable to decrease or eliminate the expression of a fatty acid destaurase encoded by at least one FAD gene and express a product of a stearoyl-ACP desaturase exogenous gene encoding an active stearoyl ACP desaturase.

73. The cell of claim 72, wherein nucleic acids are operable to decrease or eliminate the expression of a fatty acid destaurase encoded by multiple copies of a fatty acid desaturase gene.

74. The cell of claim 72 or 73, wherein the Stearoyl-ACP desaturase exogenous gene is recombined into a locus within the coding region of the fatty acid desaturase gene.

75. The cell of any of claims 72-74, wherein the oil produced has a fatty acid profile having elevated oleic acid.

76. The cell of claim 75, wherein oleic acid comprises at least 50, 60, 70, 80, or 90% of the fatty acids.

77. The cell of any of claims 46-62, wherein the recombinant cell comprises nucleic acids operable to express a product of a β-ketoacyl-ACP synthase II exogenous gene encoding an active β-ketoacyl-ACP synthase II.

78. The cell of claim 77, wherein the oil produced is characterized by a fatty acid profile elevated in C18:1 fatty acids and reduced in C16 fatty acids as a result of the recombinant nucleic acids.

79. The cell of any of claims 46-62, wherein the recombinant cell comprises nucleic acids operable to decrease or eliminate the expression of an enzyme encoded by one or more genes that encode a stearoyl ACP desaturase by RNA interference.

80. The cell according to claim 79, wherein the oil produced has a fatty acid profile characterized by an increase in C18:0 fatty acids.

81. The cell of claim 80, wherein the oil produced is characterized by a fatty acid profile having at least 50, 60, 70, 80, or 90% C18:0.

82. The cell of claim 80, wherein the oil produced is characterized by a fatty acid profile having at least 50-75% C18:0.

83. The cell of any of claims 79-82, wherein the oil produced is further characterized by a fatty acid profile having at least 20-40% C18:1.

84. The cell of any of claims 46-62, wherein the cell comprises recombinant nucleic acids operable to decrease or eliminate the expression of two copies of a gene encoding a β-ketoacyl-ACP synthase I, β-ketoacyl-ACP synthase II, stearoyl ACP desaturase, or fatty acid desaturase.

85. The cell for any of claims 46-62, operable to express a product of a fatty acid desaturase exogenous gene encoding an active a ω-3 fatty acid desaturase and/or a ω-6 oleate desaturase.

86. The cell of claim 85, wherein the oil produced has a fatty acid profile characterized by an elevated level of linoleic acid, linolenic acid, or both.

87. The cell of claim 86, wherein fatty acid profile of the oil is characterized by having at least 10, 20, 30, 40, or 50% linoleic acid, linolenic acid, or both.

88. A natural oil or oil-containing product produced from the cell of any of claims 46-87.

89. A method for producing a natural oil comprising triacylglycerides that comprise ricinoleic acid, or a product produced from the natural oil, the method comprising:

cultivating a cell of a recombinant microorganism, the cell comprising recombinant nucleic acids operable to express a product of an exogenous gene encoding an active oleate 12-hydroxylase, so as to synthesize the ricinoleic acid.

90. The method of claim 48, wherein the microorganism has a type II fatty acid biosynthesis pathway.

91. The method of claim 90, wherein the microorganism is a microalga.

92. The method of claim 91, wherein the microalga is an obligate heterotroph.

93. The method of claim 92, wherein the microalga is a species of Prototheca.

94. The method of claim 93, wherein the microalga is *Prototheca wickerhamii, Prototheca stagnora, Prototheca portoricensis, Prototheca moriformis,* or *Prototheca zopfii.*

95. The method of claim 91, wherein the microalga is *Chlorella kessleri, Chlorella luteoviridis Chlorella protothecoides,* or *Chlorella vulgaris.*

96. The method of any of claims 46-95, wherein the cell is a recombinant cell expressing an active sucrose invertase.

97. The method of any of claims 46-96, wherein the cultivating is heterotrophic.

98. The method of any of claims 46-98, wherein the cell produces at least 40, 50, 60, 70, 80, or 90% oleic acid absent the recombinant nucleic acids operable to express a product of an exogenous gene encoding an active oleate 12-hydroxylase.

99. The method of any of claims 89-98 98, further comprising recombinant nucleic acids operable to enhance oleic acid production so as to elevate the substrate levels for the oleate 12-hydroxylase.

100. The method of claim 99, wherein the cell comprises recombinant nucleic acids operable to

(a) express a product of an exogenous gene encoding an active stearoyl ACP desaturase and decrease or eliminate the expression of an enzyme encoded by one or more genes that encode a fatty acid desaturase; or

(b) express a product of an exogenous gene encoding an active β-ketoacyl-ACP synthase I and express a product of an exogenous gene encoding an active acyl-ACP thioesterase.

101. A product produced according to the method of any of claims 89-100.

102. A microorganism cell comprising recombinant nucleic acids operable to express a product of an exogenous gene encoding an active oleate 12-hydroxylase, so as to synthesize ricinoleic acid.

103. The cell of claim 102, wherein the microorganism has a type II fatty acid biosynthesis pathway.

104. The cell of claim 103, wherein the microorganism is a microalga.

105. The cell of claim 104, wherein the microalga is an obligate heterotroph.

106. The cell of claim 105, wherein the microalga is a species of Prototheca.

107. The cell of claim 106, wherein the microalga is *Prototheca wickerhamii, Prototheca stagnora, Prototheca portoricensis, Prototheca moriformis,* or *Prototheca zopfii.*

108. The cell of claim 104, wherein the microalga is *Chlorella kessleri, Chlorella luteoviridis Chlorella protothecoides,* or *Chlorella vulgaris.*

109. The cell of any of claims 102-108, wherein the cell is a recombinant cell expressing an active sucrose invertase.

110. The cell of any of claims 102 to 109 , wherein the cell is capable of heterotrophic growth.

111. The cell of any of claims 102 to 109, wherein the cell produces at least 40, 50, 60, 70, 80, or 90% oleic acid absent the recombinant nucleic acids operable to express a product of an exogenous gene encoding an active oleate 12-hydroxylase.

112. The cell of claim any of claims 102-111, further comprising recombinant nucleic acids operable to enhance oleic acid production so as to elevate the substrate levels for the oleate 12-hydroxylase.

113. The cell of claim 112, wherein the cell comprises recombinant nucleic acids operable to

(a) express a product of an exogenous gene encoding an active stearoyl ACP desaturase and decrease or eliminate the expression of an enzyme encoded by one or more genes that encode a fatty acid desaturase; or

(b) express a product of an exogenous gene encoding an active β-ketoacyl-ACP synthase I and express a product of an exogenous gene encoding an active acyl-ACP thioesterase.

114. A food comprising an oil of claim 44.

CLAIMS:

1. A method for using sucrose to produce an oil comprising triacylglycerides that comprise ricinoleic acid, or a product produced from the oil, the method comprising:

cultivating a cell of a recombinant oleaginous microalga of the species *Prototheca moriformis*, the cell comprising:

nucleic acids operable to enhance oleic acid production so as to elevate the substrate levels for an exogenous oleate 12-hydroxylase by expressing a product of an exogenous gene encoding an active stearoyl ACP desaturase and decreasing or eliminating the expression of an enzyme encoded by one or more endogenous genes that encode a fatty acid desaturase by directly targeting said endogenous gene(s) or RNA products of said gene(s); and

an exogenous nucleic acid operable to express the active oleate 12-hydroxylase, so as to synthesize the ricinoleic acid; and an exogenous nucleic acid operable to express an active invertase; and

feeding sucrose to the cell so as to produce the oil comprising triacylglycerides comprising ricinoleic acid, wherein the ricinoleic acid is detectable by gas chromatography-mass spectrometry.

2. The method of claim 1, wherein the cell produces at least 40% oleic acid.

3. A microalga cell of the species *Prototheca moriformis*, the cell comprising:

nucleic acids operable to enhance oleic acid production so as to elevate the substrate levels for an exogenous oleate 12-hydroxylase by expressing a product of an exogenous gene encoding an active stearoyl ACP desaturase and decreasing or eliminating the expression of an enzyme encoded by one or more endogenous genes that encode a fatty acid desaturase by directly targeting said endogenous gene(s) or RNA products of said gene(s);

an exogenous gene encoding the active oleate 12-hydroxylase; and

an exogenous gene encoding an invertase so as to enable the cell to use sucrose to synthesize ricinoleic acid in an amount detectable by gas chromatography-mass spectrometry.

4. The method of claim 1, wherein the oleate 12-hydroxylase is from *Ricinus communis*, *Physaria lindheimeri*, or *Arabidopsis lyrata*.

5. The method of claim 1, wherein the oleate 12-hydroxylase has an amino acid sequence corresponding to any of SEQ ID NOs 259-262.

6. The method of claim 3, wherein the oleate 12-hydroxylase is from *Ricinus communis*, *Physaria lindheimeri*, or *Arabidopsis lyrata*.

7. The method of claim 3, wherein the oleate 12-hydroxylase has an amino acid sequence corresponding to any of SEQ ID NOs 259-262.